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(54) PROTEIN D - AN IgD-BINDING PROTEIN OF HAEMOPHILUS INFLUENZAE

PROTEIN D- EIN IGD-BINDENDES PROTEIN VON HAEMOPHILUS INFLUENZAE

PROTEINE D - PROTEINE FIXATRICE D'IgD, DE HAEMOPHILUS INFLUENZAE

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- **Infection and Immunity, Vol. 59, No. 1, January 1991 HAKAN JANSON et al.: "Protein D, an Immunoglobulin D-Binding Protein of Haemophilus influenzae: Cloning, Nucleotide Sequence, and Expression in Escherichia coli", see page 119 - page 125.**
- **The Journal of Immunology, Vol. 145, November 1990 MAORONG RUAN et al.: "Protein D of Haemophilus influenzae A Novel Bacterial Surface Protein withAffinityfor Human IgD" see page 3379 - 3384**
- **The Journal of Immunology, Vol. 122, No. 4, April 1979 ARNE FORSGREN et al.: "Many bacterial species bind human IgD", see page 1468 - page 1472.**

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EP 0 594 610 B1

Description

The present invention is related to a surface exposed protein named protein D which is conserved in many strains of Haemophilus influenzae or related Haemophilus species. Protein D is an Ig receptor for human IgD.

Several immunoglobulin (Ig) binding bacterial cell wall proteins have been isolated and/or cloned during the last two decades. The best characterized of these are protein A of Staphylococcus aureus and protein G of group G beta-hemolytic streptococci. The classical Fc-binding capacity of protein A involves IgG from humans and several mammalian species but the binding is restricted to human IgG subclasses 1, 2 and 4. Also other human classes of Ig (G, A, M, E) have been shown to bind to protein A, a reactivity that has been designed the alternative Ig binding which is mediated by Fab structures and characterized by a variable occurrence in the different Ig classes.

Protein G of group G streptococci binds all human IgG subclasses and has also a wider binding spectrum for animal IgG than protein A. On the IgG molecule the Fc part is mainly responsible for the interaction with protein G although a low degree of interaction was also recorded for Fab fragments. IgM, IgA and IgD, however, show no binding to protein G. Both protein A and protein G have acquired many applications for immunoglobulin separation and detection. (EP 0 200 909, EP 0 131 142, WO 87/05631, US 3,800,798, US 3,995,C18.)

Certain strains of group A streptococci are also known to produce an IgG-binding protein which has been purified or cloned. The Ig-binding protein from group A streptococci is relatively specific for human IgG. Information about bacterial molecules that selectively bind IgA and IgM is more limited. However, IgA-binding proteins have been isolated from both group A and group B streptococci, two frequent human pathogens. The IgA receptor of group A streptococci has been named protein Arp. Certain strains of the anaerobic bacterium Clostridium perfringens preferentially bind IgM but also IgA and IgG. This binding is due to a cell surface protein (protein P). Recently a bacterial protein, protein L, with unique binding properties for L-chains was isolated from Peptococcus magnus. Protein L has been shown to bind IgG, IgA and IgM from human and several mammalian species. Among gram-negative bacteria, Ig receptors have been reported among veterinary pathogens. Brucella abortus binds bovine IgM and Taylorella equigenitalis, a venereal pathogen of horses, binds equine IgG. Recently Haemophilus somnus was reported to bind bovine IgG.

A decade ago Haemophilus influenzae and Moraxella (Branhamella) catarrhalis were shown to have a high binding capacity for human IgD (Forsgren A. and Grubb A, J. Immunol. 122:1468, 1979).

The present invention describes the solubilization and purification of a H. influenzae surface protein responsible for the interaction with IgD. It also describes the cloning, expression and nucleotide sequence of the IgD-binding protein gene of the H. influenzae in Escherichia coli. In addition it describes the Ig-binding properties of this molecule, named protein D, which were found to be different compared with previously isolated Ig-binding proteins. Protein D was found only to interact with IgD and not with other human immunoglobulin classes. Thus, protein D could be an important tool for studies, separation and detection of IgD in a way similar to the way in which protein A and protein G previously have been used for IgG. Protein D could also be a valuable tool alone and in combination with other molecules (for example proteins and polysaccharides) in the stimulation of the immune system through an interaction with B-lymphocytes. Protein D is not identical with any previously described protein from H. influenzae.

H. influenzae is a common human parasite and pathogen which colonizes the mucosa of the upper respiratory tract and causes disease by local spread or invasion. An important distinguishing feature between H. influenzae isolates is whether or not they are encapsulated. Encapsulated H. influenzae type b is a primary cause of bacterial meningitis and other invasive infections in children under 4 years of age in Europe and the United States. Non-encapsulated (non-typable) H. influenzae rarely cause invasive infection in healthy children and adults but are a frequent cause of otitis media in children and have been implicated as a cause of sinusitis in both adults and children. H. influenzae are also commonly isolated in purulent secretions of patients with cystic fibrosis and chronic bronchitis and have recently been recognized as an important cause of pneumonia.

A vaccine composed of purified type b capsular polysaccharide has proven effective against H. influenzae type b disease in children of 2 to 5 years of age. However, since children under two years of age respond poorly to this vaccine, conjugate vaccines with enhanced immunogenicity have been developed by covalently bonding the capsular polysaccharide to certain proteins. However, the polysaccharide vaccines, nonconjugated and conjugated, are of no value against nontypable H. influenzae disease. Hence, other cell surface components and in particular outer membrane proteins (OMPs) have been looked at as potential vaccine candidates both against type b and nontypable H. influenzae. (EP 0 281 673, EP 0 320 289.)

The outer membrane of H. influenzae is typical of gram-negative bacteria and consists of phospholipids, lipopolysaccharide (LPS), and about 24 proteins. Four different Haemophilus OMPs have been shown to be targets for antibodies protective against experimental Haemophilus disease. These include the P1 heat-modifiable major outer membrane protein, the P2 porin protein, the P6 lipoprotein and a surface protein with an apparent molecular weight of 98,000 (98 K protein). Of these at least antibodies to P2 have been shown not to

protect against challenge with heterologous Haemophilus strains. (Loeb, M. R. Infect. Immun. 55:2612, 1987; Munson Jr, R. S. et al J. Clin. Invest. 72:677, 1983; Munson Jr, R. S. and Granoff, D. M. Infect. Immun. 49:544, 1985 and Kimura, A. et al, Infect. Immun. 194:495, 1985).

Analysis of nontypable H. influenzae has shown that there are marked differences in OMP composition among strains (See e.g. Murphy et al. "A subtyping system for nontypable Haemophilus influenzae based on outer membrane proteins" J Infect Dis 147:838, 1983; Barenkamp et al. "Outer membrane protein and biotype analysis of pathogenic nontypable Haemophilus influenzae" Infect Immun 30:709, 1983).

If a surface exposed antigen (immunogen) which is conserved in all strains of H. influenzae could be found it would be an important tool in developing a method of identifying H. influenzae in clinical specimens as well as a vaccine against H. influenzae. The present invention shows that protein D with an identical apparent molecular weight (42,000), reacting with three different monoclonal antibodies and human IgD, was found in all 116 H. influenzae strains (encapsulated and nonencapsulated) studied, as well as in two other related Haemophilus species, namely H. haemolyticus and H. aegypticus.

Thus, according to the invention there is provided a surface exposed protein, which is conserved in many strains of Haemophilus influenzae or related Haemophilus species, having an apparent molecular weight of 42,000 and a capacity of binding human IgD. The invention also comprises naturally occurring or artificially modified variants of said protein having the same function as said protein, and also immunogenic or IgD-binding portions of said protein and variants. The protein is named protein D and has the amino acid sequence depicted in Fig. 9.

There is also provided a plasmid or phage containing a genetic code for protein D or the above defined variants or portions.

Further there is provided a non-human host containing the above plasmid or phage and capable of producing said protein or variants, or said portions thereof. The host is chosen among bacteria, yeasts or plants. A presently preferred host is E. coli.

In a further aspect the invention provides for a DNA segment comprising a DNA sequence which codes for protein D, or said variants thereof, or for said portions. The DNA sequence is shown in Fig. 9.

In yet another aspect, the invention provides for a recombinant DNA molecule containing a nucleotide sequence coding for protein D, or said variants or portions, which nucleotide sequence could be fused to another gene.

A plasmid or a phage containing the fused nucleotide defined above could also be constructed.

Further such a plasmid or phage could be inserted in a non-human host, such as bacteria, yeasts or plants. At present, E. coli is the preferred host.

The invention also comprises a fusion protein or polypeptide in which protein D, or said variants or portions, could be combined with another protein by the use of a recombinant DNA molecule, defined above.

Furthermore, a fusion product in which protein D, or said variants or portions, is covalently or by any other means bound to a protein, carbohydrate or matrix (such as gold, "Sephadex" particles, polymeric surfaces) could be constructed.

The invention also comprises a vaccine containing protein D, or said variants or portions. Other forms of vaccines contain the same protein D or variants or portions, combined with another vaccine, or combined with an immunogenic portion of another molecule.

There is also provided a hybridoma cell capable of producing a monoclonal antibody specific to an immunogenic portion of protein D, or of naturally occurring or artificially modified variants thereof, with the proviso that the antibody is not IgD.

Further there is provided a purified antibody which is specific to an immunogenic portion of protein D or of naturally ooccuring or artificially modified variants thereof, with the proviso that the antibody is not IgD. This antibody is used in a method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with the antibody in the presence of an indicator.

The invention also comprises a method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with a DNA probe or primer constructed to correspond to the nucleic acids which code for protein D, or for naturally occurring or artificially modified variants thereof, or for an immunogenic or IgD-binding portion of said protein or variants.

Protein D, or said variants or portions, is also used in a method of detecting IgD. In such a detecting method the protein may be labelled or bound to a matrix.

Finally, the invention comprises a method of separating IgD using protein D, or said variants or portions, optionally bound to a matrix.

MATERIALS AND METHODS

Bacteria

116 H. influenzae strains representing serotypes a-f and nontypable and in addition bacterial strains representing 12 species related to H. influenzae were obtained from different laboratories in Denmark, Sweden and the U.S.A.

Culture conditions

All strains of Haemophilus, Ekinella and Acinobacillus were grown on chocolate agar. H. ducreyi were grown in microaerophilic atmosphere at 37°C and all

other Haemophilus strains in an atmosphere containing 5% CO_2. 30 isolates of H. influenzae were also grown overnight at 37°C in brain-heart infusion broth (Difco Lab., Inc. Detroit, Mi.) supplemented with nicotinamide adenine dinucleotide and hemin (Sigma Chemical Co. St Louis, Mo.), each at 10 pg/ml.

Immunoglobulins and proteins

IgD myeloma proteins from four different patients were purified as described (Forsgren, A. and Grubb, A., J. Immunol. 122:1468, 1979). Eight different human IgG myeloma proteins representing all four subclasses and both L-chain types, three different IgM myeloma proteins and one IgA myeloma protein were isolated and purified according to standard methods. Human polyclonal IgG, serum albumin and plasminogen were purchased from Kabi Vitrum AB, Stockholm, Sweden, and human IgE was adapted from Pharmacia IgE RIACT kit (Pharmacia Diagnostic AB, Uppsala, Sweden). Bovine serum albumin, human and bovine fibrinogen and human transferrin were purchased or obtained as a gift.

^{125}I-IgD binding assay

The binding assay was carried out in plastic tubes. Briefly $4x10^8$ bacterial cells in a volume of 100 µl phosphate buffered saline (PBS) with the addition of 5% human serum albumine (HSA) were mixed with 100 µl of ^{125}I-IgD in the same buffer (radioactivity was adjusted to 7-$8x10^4$ cpm, i.e approx. 40 ng). After 0.5 h incubation at 37°C, 2 ml of ice-cold PBS (containing 0.1% Tween 20) was added to the tubes.

The suspension was centrifugated at 4,599xg for 15 min and the supernatant was aspirated. Radioactivity retained in the bacterial pellet was measured in a gamma counter (LKB Wallac Clingamma 1271, Turku, Finland). Residual radioactivity from incubation mixtures containing no bacteria, i.e. background, was 2.5 percent. Samples were always tested in triplicates and each experiment was repeated at least twice, unless otherwise stated.

Monoclonal antibodies

Inbred female BALB/c mice (age 8 to 14 weeks) were immunized by an intraperitoneal injection of 25 µg purified protein D (25 pg/50 p1) in Freund's complete adjuvant (300 µl) followed by two intraperitoneal injections of protein D (15 pg) in Freund's incomplete adjuvant (300 µl) 3 and 7 weeks later. In week 9 the mice were bled from the tails, serum was separated and tested for anti-protein D activity in an enzyme-linked immunosorbent assay (ELISA). The best responding mouse was boosted by an intravenous injection of protein D (2 µg) in 150 µl PBS. One day after the last injection, the spleen was excised and spleen cells were prepared for the production of monoclonal antibodies (De St Groth SF, Scheidegger SJ J Immunol Methods 35:1, 1980). After 10 to 14 days (mean 12 days) the hybridomas were tested for the production of antibodies against protein D in an enzyme-linked immunosorbent assay (ELISA), and the hybrids producing the highest titers of antibodies were cloned and expanded by cultivation in RPMI medium containing 10% fetal bovine serum. Totally 68 clones producing antibodies to protein D were obtained. Three of the hybridomas were selected for further growth in the same medium. All cell lines were frozen in the presence of dimethyl sulfoxide and 90% fetal bovine serum in liquid nitrogen.

SDS-PAGE and detection of protein D on membranes

SDS-PAGE was, using a modified Laemmli gel, prepared and run according to the procedure of Lugtenberg et al., (FEBS Lett 58:254, 1975) using a total acrylamide concentration of 11%. Samples of crude Sarcosyl extracts of H. influenzae and related bacterial species were pretreated by 5-min boiling in sample buffer consisting of 0.06M of Tris hydrochloride (pH 6.8), 2% (w/v) SDS, 1% (v/v) β-ME, 10% glycerol, and 0.03% (w/v) bromphenol blue. Electrophoresis was performed at room temperature using PROTEIN II vertical slab electrophoresis cells (Bio-Rad Laboratories, Richmond, CA) at 40 mA per gel constant current. Staining of proteins in gels was done with comassie brilliant blue in a mixture of methanol, acetic acid and water essentially as described by Weber and Osborn (J. Biol. Chem. 244:4406, 1969). Protein bands were also transferred to nitrocellulose membranes (Sartorius, West Germany) by electrophoretic transfer from SDS-polyacrylamide gels. Electrophoretic transfer was carried out in a Trans-Blot Cell (Bio-Rad) at 50 V for 90 min. The electrode buffer was 0.025M Tris, pH 8.3, 0.192M glycine, and 20% methanol. The membranes were then washed for 1 h at room temperature in 1.5% ovalbumin-Tris balanced saline (OA-TBS), pH 7.4, to saturate additional binding sites.

After several washings with Tris balanced saline (TBS), the membranes were incubated overnight at room temperature in 1% OA-TBS buffer containing IgD (20 pg/ml) to detect IgD-binding bands, then washed twice with TBS. The membranes were then incubated with peroxidase conjugated goat anti-human IgD (Fc) (Nordic Immunology, Tiiburg, The Netherlands) for 1-2 hrs at room temperature; after several washings with Tween-TBS the membranes were developed with 4-chloro-1-napthol and hydrogen peroxide. Protein D was also identified using anti-protein D mouse monoclonal antibodies 16ClO, 20G6 and 19B4 at 1:50 dilution in 1% OA-TBS. Protein 1 and 2 of H. influenzae were identified using anti-P2 mouse monoclonal 9F5 (Dr. Eric J. Hansen, Dallas, Texas, USA) at a 1:1000 dilution and rabbit anti-PI serum (Dr. Robert S. Munson, St. Louis, Mo, USA) at a 1:200 dilution.

Solubilization and purification of protein D from H. influenzae

Briefly 3 g of bacteria were suspended in 10 ml of 10 mM HEPES Tris buffer (pH 7.4) containing 0.01M EDTA and sonicated three times in a sonifier (MSE) for 1 min while cooling in an ice bath. Following sonication Sarcosyl (Sodium Lauryl Sarcosinate) was added to a final concentration of 1% (w/v). The suspensions were incubated at room temperature for 1 h using a shaker and then sonicated again 2x1 min on ice and reincubated at room temperature for 30 min. After centrifugation at 12,000 g for 15 min at 4°C the supernatant was harvested and recentrifugated at 105,000 g for 1.5 h at 4°C.

Sarcosylextracts prepared of H. influenzae, strain NT 772 as described above were applied to SDS-PAGE. After electrophoresis narrow gel strips were cut out, protein was transferred to membranes and the IgD-binding band was detected by Western blot assay using IgD and peroxidase conjugated goat anti-human IgD as described above (see SDS-PAGE and detection of protein D on membranes). By comparison with the IgD-binding band on the membrane (Western blot) the appropriate band in the gel could be identified and cut out. Electrophoretic elution of the IgD-binding molecules (protein D) was performed and SDS was removed from the protein containing solution by precipitation in potassium phosphate buffer using a method from Susuki and Terrada (Anal. Biochem. 172:259, 1988). Potassium phosphate in a final concentration of 20 mM was added and after incubation at 4°C overnight the SDS-precipitate was removed by centrifugation at 12,000 g. Thereafter the potassium content was adjusted to 60 mM and after 4 hrs at 4°C centrifugation was performed as above. Finally the supernatant was concentrated and extensive dialysis was performed.

Dot blot assay

Proteins were applied to nitrocellulose membranes (Schleicher & Schuell, Dessel, West Germany) manually by using a dot blot apparatus (Schleicher & Schuell). After saturation, the membranes were incubated overnight at room temperature in 1% OA-TBS containing ^{125}I-labeled protein probe (5 to 10x10^5 cpm/ml), washed four times with TBS containing 0.02% Tween-20, air dried, and autoradiographed at -70°C by using Kodak CEA.C X-ray films and Kodak X-Omat regular intensifying screen (Eastman Kodak, Rochester, NY).

Amino acid sequence analysis

Automated amino acid sequence analysis was performed with an Applied Biosystems 470A gas-liquid solid phase sequenator (A) with online detection of the released amino acid phenylthiohydantoin derivatives by Applied Biosystems Model 120A PTH Analyzer.

Bacterial strains, plasmids, bacteriophages and media used for cloning of protein D

H. influenzae, nontypable strain 772, biotype 2, was isolated from a nasopharyngeal swab at the Department of Medical Microbiology, Malmö General Hospital, University of Lund, Sweden. E. coli JM83 were used as recipient for plasmids pUC18 and pUC19 and derivatives thereof. E. coli JM101 and JM103 were used as hosts for M13mp18 and mp19 bacteriophages. H. influenzae was cultured in brain-heart infusion broth (Difco Lab., Inc. Detroit, Mi.) supplemented with NAD (nicotine adenine dinucleotide) and hemin (Sigma Chemical Co., St Louis, Mo.), each at 10 µg/ml. E. coli strains were grown in L broth or 2xYT media. L agar and 2xYT agar contained in addition 1.5 g of agar per litre. L broth and L agar were, when so indicated, supplemented with ampicillin (Sigma) at 100 µg/ml.

DNA preparations

Chromosomal DNA was prepared from H. influenzae strain 772 by using a modification of the method of Berns and Thomas (J Mol. Biol. 11:476, 1965). After the phenol:chloroform:isoamylalcohol (25:24:1) extraction step the DNA was ethanol precipitated. The DNA was dissolved in O.lxSSC (1xSSC:0.15 M NaCl and 0.015 M sodium citrate) and RNase treated for 2 h at 37°C. The RNase was removed with two chloroform:isoamylalcohol (24:1) extractions. The DNA was banded in a CsCl-ethidium bromide equilibrium gradient.

Plasmid DNA and the replicative form of phage M13 from E. coli JM101 were obtained by the alkaline lysis procedure followed by further purification in a CsCl- -ethidium bromide gradient. In some cases plasmid DNA was prepared using a Quiagen plasmid DNA kit (Diagen GmbH Düsseldorf, FRG).

Single-stranded (ss) DNA from phage M13 clones was prepared from single plaques (Messing, J. Meth. Enzymol 101C:20, 1983).

Molecular cloning of the protein D gene

A H. influenzae genomic library was constructed starting from 40 µg of H. influenzae strain 772 DNA which was partially digested with 1.2 units Sau3A for 1 h at 37°C. The cleaved DNA was fractionated on a sucrose gradient (Clark-Curtiss, J. E. et al., J. Bacteriol. 161:1093, 1985). Fractions containing DNA fragments of appropriate sizes (2-7 kilobasepairs (kbp)) were pooled and the DNA was ligated to dephosphorylated BamHI digested pUC18 under standard conditions (Maniatis, T. et al., Molecular cloning: A laboratory manual, 1982). The ligation mixture was transformed into component E. coli JM83 by high voltage electroporation with a Gene Pulser ™/Pulse controller apparatus, both from Bio-Rad Lab. (Richmond, CA). The bacteria were plated onto L agar supplemented with ampicillin and X-gal

(5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside).

Colony immunoassay

For colony immunoblotting, E. coli transformants, cultivated overnight on L agar, were transferred to nitrocellulose filters (Sartorius GmbH, Göttingen, FRG) by covering the agar surfaces with dry filters. The plates were left for 15 min before the filters were removed and exposed to saturated chloroform vapour for 15 min. Residual protein binding sites on the filters were blocked by incubating the filters in Tris balanced saline containing ovalbumine for 30 min (TBS-ova; 50 mM Tris-HCl, 154 mM NaCl, 1.5% ova.; pH 7.4). After blocking, the filters were incubated in turn with (i) culture supernatants containing mouse monoclonal antibodies (MAbs) directed against protein D at a dilution of 1:10 in TBS-ova, (ii) horseradish peroxidase conjugated rabbit anti-mouse IgGs (DAKOPATTS A/S, Glostrup, Denmark) in TBS-ova at a dilution of 1:2000 in TBS-ova, and (iii) 4-chloro-1-naphthol and H_2O_2. The filters were washed 3x10 min in wash buffer (TBS-0.05% Tween 20) between each step. All incubations were done at room temperature.

Colonies were also checked for IgD binding by incubating other filters with purified human myeloma IgD: s, rabbit anti-human IgD (6-chains) (DAKOPATTS), horseradish peroxidase conjugated goat anti-rabbit Ig: s (Bio-Rad Lab.) and 4-chloro-1-naphthol and H_2O_2 as above.

Restriction endonuclease analysis and DNA manipulations

Plasmid and phage DNA were digested with restriction endonucleases according to the manufacturers' instructions (Boehringer Mannheim mbH, Mannheim, FRG, and Beckman Instruments, Inc., England). Restriction enzyme fragments for subcloning were visualised with low energy UV-light and excised from 0.7-1.2% agarose gels (Bio-Rad) containing 0.5% ethidium bromide. The DNA bands were extracted with a Geneclean ™ kit (BIO 101 Inc., La Jolla, Ca.) as recommended by the supplier.

Ligations were performed with 14 DNA ligase (Boehringer Mannheim) under standard conditions (Maniatis et al., 1982). The ligation mixtures were used to transform competent E. coli cells.

Progressive deletions of the recombinant plasmid pHIC348 for the sequencing procedure were produced by varying the time of exonuclease III digestion of KpnI-BamHI-opened plasmid DNA (Henikoff, S. Gene 28: 351, 1984). For removal of the resulting single-stranded ends, mung bean nuclease was used. Both nucleases were obtained from Bethesda Research Laboratories Inc. (Gathersburg, Md.).

Protein D extraction from E. coli

Cells of E. coli expressing protein D were grown in L broth supplemented with ampicillin to early logarithmic phase and then subjected to osmotic shock. After removal of periplasmic fraction the cells were lysed with NaOH (Russel, M. and Model, P., Cell 28:177, 1982) and the cytoplasmic fraction was separated from the membrane fraction by centrifugation. The periplasmic and cytoplasmic proteins were precipitated with 5% tri-chloro acetic acid.

DNA sequencing and sequence manipulations

The nucleotide sequence was determined by direct plasmid sequencing (Chen, E. Y. and Seeburg, P. H. DNA 4:165, 1985) of subclones and deletion derivatives of plasmid pHIC348 using the chain termination method with α[^{35}S]-dATP (Amersham) and Sequenase™, version 2 (United States Biochemical Corp., Cleveland, Ohio) following the protocol provided by the supplier. Part of the sequencing was done on single-stranded M13 DNA carrying inserts derived from pHIC348. Autoradiography was performed with Fuji X-ray film.

RESULTS

Distribution of protein D in Haemophilus influenzae

A total of 116 H. influenzae strains obtained from culture collections and freshly isolated from nasopharyngeal swabs were selected for IgD-binding experiments. Eleven of the strains were encapsulated representing serotypes a-f, and 105 strains were non-encapsulated (nontypable). These 105 strains belonged to biotype I (21 strains), biotype II (39 strains), biotype III (14 strains), biotype IV (2 strains) and biotype I (5 strains). Of the non-encapsulated strains 31 were not biotyped (NBT) but tested for IgD binding.

Approximately 4x10^8 cfu of H. influenzae bacteria grown on chocolate agar were mixed and incubated with 40 ng of radiolabeled human myeloma IgD. Thereafter a larger volume (2 ml) of PBS containing Tween 20 was added, bacteria were spun down and radioactivity of pellets was measured. All H. influenzae isolates bound IgD to a high degree (38-74%) (Fig. 1). There was no difference in IgD-binding capacity between different serotypes (a-f) of encapsulated H. influenzae. Nor was there any difference between different biotypes of non-encapsulated strains. 30 strains representing different sero- and biotypes were also grown in brain-heart infusion broth. When those bacteria grown in liquid medium were compared with the same bacteria grown on chocolate agar, no difference in IgD-binding capacity could be detected.

Protein D was solubilized from all 116 H. influenzae strains by sonication and Sarcosyl extraction. Subsequently the extracts containing protein D were subjected

to SDS-PAGE. Proteins were stained or electroblotted onto nitrocellulose membranes and probed with human IgD myeloma protein and three different mouse monoclonal antibodies recognizing protein D. Many protein bands could be detected in all SDS-gels but electrophoresis of extracts from all H. influenzae isolates gave a protein band with an apparent molecular weight of 42,000 (42 kilodaltons). IgD and also all three anti-protein D monoclonal antibodies (16C10, 20G6 and 19B4) bound to the same band after electrophoresis of all extracts and subsequent transfer to membranes and blotting.

Bacterial strains of 12 different species taxonomically related to H. influenzae (H. ducreyi, H. paraphrophilus, H. parasuis, H. parainfluenzae, H. haemolyticus, H. parahaemolyticus, H. aphrophilus, H. segnis, H. aegypticus, H. haemoglobinophilus, E. corrodens, A. actinomycetemcomitans) were tested for their capacity to bind ^{125}I labeled human IgD. In addition crude Sacrosyl extracts from the same bacteria were tested by Western blot analysis with IgD and the three anti-protein D monoclonal antibodies (MAbs 16C10, 20G6, 19B4).

Of all twelve species tested, only H. haemolyticus (5/5 strains) and H. aegypticus (2/2 strains) bound radiolabeled IgD, 21-28% and 41-48%, respectively, in the direct binding assay (Fig. 2). In Western blot analysis IgD and all three monoclonal antibodies detected a single band with an apparent molecular weight of 42,000 (42 kilodaltons).

None of the 6 strains of H. paraphrophilus, 11 H. parainfluenzae, 8 H. aphrophilus, and 3 A. actinomycetemcomitans bound radiolabeled IgD in the direct binding assay or reacted with IgD in Western blot analysis. However, extracts of all these strains reacted with two or three of the monoclonal antibodies in Western blot analysis showing a single 42 kilodaltons protein band. Western blot analysis of three strains of E. corrodens revealed a single high molecular weight band (90 kilodaltons) with MAb 16C10 in all three strains. In an extract of one of the strains, a single 42 kilodaltons band was detected with the two other monoclonal antibodies. Two strains of H.ducreyi, H. parasuis (2 strains), H. parahaemolyticus (2 strains), H. sengius (2 strains), H. haemoglobinophilus (1 strain) did not bind radiolabeled IgD in the direct binding assay and Sarcosyl extracts from the same bacteria did not reveal any protein band detectable by IgD or the three monoclonal antibodies.

Solubilization of protein D

Three different strains of H. influenzae (two nontypable strains, 772 and 3198 and one type B, Minn A.) were grown overnight in broth. Initially attempts were made to solubilize protein D according to a well established method for isolation of H. influenzae outer membrane proteins by sonication, removal of the cell debris by centrifugation and extraction of the supernatant with Sarcosyl followed by ultracentrifugation (Barenkamp SJ and Munson RS J Infect Dis 143:668, 1981). The pellets (cell debris) (d) and supernatants (s) after sonication as well as the pellets (p) and supernatants (ss) after Sarcosyl-treatment and ultracentrifugation were subjected to SDS-PAGE. Proteins were stained or electroblotted onto Immobilon membranes and probed with human IgD myeloma protein followed by incubation with peroxidase conjugated anti-human IgD-antibodies and substrate. As shown in Fig. 3 the sonication procedure solubilized proteins including protein D effectively. However, IgD-binding molecules (protein D) could also be detected in the cell debris, i.e. were not solubilized by sonication. The yield of IgD-binding molecules in the supernatant varied between different experiments. Fig. 3 also shows that protein D mostly could be detected in the Sarcosyl soluble supernatant after ultracentrifugation. In contrast previously described outer membrane proteins of H. influenzae (protein 1 to 6) are readily solubilized by sonication and are considered Sarcosyl insoluble.

To improve the yield of protein D several extraction methods were tried. In subsequent experiments the bacterial cells were sonicated and the whole cell suspension sonicated and extracted in different detergents (Sarcosyl, NP-40, Triton X-100 and Tween 80). The cell debris was removed by centrifugation (12,000 g) and the supernatant ultracentrifuged. The thus obtained cell debris (d), supernatants (s) and pellets (p) were analysed by SDS-PAGE, electroblotting onto membranes and subsequent probing with IgD. As shown in Fig. 4 Sarcosyl treatment effectively solubilized protein D leaving little left in the cell debris and pellet. NP-40, Triton X-100 and Tween-80 solubilized protein D less effectively.

Attempts were also made to solubilize protein D from the bacteria with lysozyme and different proteolytic enzymes (papain, pepsin and trypsin) at different concentrations. Of the enzymes only lysozyme solubilized protein D (Fig. 4).

Purification of protein D

Protein D was solubilized by Sarcosyl extraction of whole bacteria as described above and purification was performed by SDS-PAGE of the supernatant after ultracentrifugation. After electrophoresis narrow gel strips were cut out, proteins were transferred to membranes and the IgD-binding band (protein D) was detected by Western blot assay. Gel slices containing a protein band corresponding to the IgD-binding molecules were cut out from the gel and solubilized by electronic elution. At reelectrophoresis the purified protein, protein D (D), migrated as a single band (42 kilodaltons) (Fig. 5) without discernible breakdown products.

To confirm that protein D was not identical with the previously described outer membrane proteins 1 or 2 with molecular weights of 49 and 39 kilodaltons, respectively, debris (d) and supernatants (s) after Sarcosyl extraction of whole H. influenzae bacteria were subjected

to SDS-PAGE, transferred to Immobilon filters and blotted with antibodies to protein 1 and protein 2 and also with human IgD. As can be seen in Fig. 5 protein D migrates differently from protein 1 and protein 2.

Binding properties of protein D

The interaction of protein D with human IgD was further verified in gel filtration experiments where ^{125}I- -protein D was eluted together with IgD when a mixture of the two proteins was run on a Sephadex G-200 column (Fig. 6c). Protein D run alone on the same column was eluted slightly after the 43 kilodaltons standard protein (Ovalbumin) confirming the apparent molecular weight of 42 kilodaltons for protein D.

Radiolabeled protein D was also studied in different dot blot experiments to further examine the binding specificity of the molecule. Fig. 7 shows that protein D effectively bound two highly purified human IgD myeloma proteins. A distinct reaction could be detected at 0.15 and 0.3 µg of the two IgD proteins, respectively. Two additional IgD myeloma proteins which were tested with the same technique could also distinctly be detected at 0.3 µg (data not shown). In dot blots IgD-Fab fragments and IgD- -Fc fragments bound protein D at 2.5 and 1.2 µg, respectively. In contrast 8 different IgG myeloma proteins representing all subclasses and L-chain types showed no visible reaction with protein D at 5 µg. Neither could any reaction between protein D and three monoclonal IgM, one monoclonal IgA preparation, polyclonal IgE or some additional proteins be detected. However, with polyclonal IgG a weak reaction was detected at 5 µg (Fig. 7).

Cloning of the protein D gene

DNA isolated from H. influenzae 772 was partially digested with Sau3A and enriched for fragments in the size of 2 to 7 kilobasepairs (kbp) by fractionation on a sucrose gradient. These fragments were ligated to the BamHI-cut and phosphatase-treated vector pUC18. E. coli JM83 cells transformed with the ligation mixture by high voltage electroporation were plated selecting for resistance to ampicillin. Individual colonies were transferred to nitrocellulose filters and screened with a cocktail of monoclonal antibodies (MAbs) as described in **Materials and Methods**

Among the 15,000 colonies tested, 60 were found positive. Eight positive colonies were picked, purified and subjected to another two rounds of screening. All clones remained positive during the purification. The purified clones were tested for IgD binding with human IgD, rabbit anti-human IgD and peroxidase conjugated goat anti-rabbit Ig:s in a colony immunoassay as described in Materials and Methods. All were positive regarding IgD binding. Additionally, the clones were found positive when screening with the three MAbs individually.

Restriction enzyme analysis of plasmid DNA from the positive clones showed that all but one clone carried a 3.3 kbp insert with two internal Sau3A sites. One clone contained an additional 2.0 kbp Sau3A fragment. One of the smaller recombinant plasmids, pHIJ32, was chosen for further characterization. A partial restriction enzyme map was established for the insert of H. influenzae DNA in pHIJ32 (Fig. 8). To identify the region coding for protein D, restriction enzyme fragments were subcloned into pUC18. The resulting transformants were tested for expression of protein D using colony immunoblot analysis as described above. These experiments showed that plasmids carrying a 1.9 kbp HindIII-ClaI fragment from one end of the insert allowed expression of IgD-binding protein. This recombinant plasmid, called pHIC348, was kept for further experiments. The protein D gene cloned in pHIC348 is expressed from a promoter in pUC18. This was shown by cloning the HindIII-ClaI fragment of pHIJ32 in the opposite orientation in pUC19. All transformants expressed IgD binding, as would be expected if the gene is under the control of an endogenous promoter. Transformants carrying the HindIII-ClaI fragment in the opposite direction to pHIC348 grew poorly and autolysed during cultivation. This was probably due to the lacZ promoter of pUC19 being oriented in the same direction as the promoter of protein D which led to an overexpression of protein D which was lethal to the cells. In pHIC348 the lacZ promoter was in the opposite direction of the protein D promoter.

DNA sequence analysis of the protein D gene

The nucleotide sequence of both strands of the insert from pHIC348 was determined either by direct plasmid sequencing of subclones and deletion constructs or by subcloning restriction fragments into phages M13mp18 and M13mp19. Commersially available universal and reverse M13 primers were used. Sequencing was done across all restriction enzyme sites used in subcloning and the sequencing strategy is outlined in Fig. 8.

The DNA sequence (Fig. 9) reveals an open reading frame of 1092 bp starting with an ATG codon at position 204 and finishing at position 1296 with a TAA stop codon. The open reading frame corresponds to a protein of 364 amino acid residues. Ten nucleotides upstream of the methionine codon is a sequence, AAGGAG, that is complementary to the 3' end of the 16S rRNA of E. coli (Shine, J. and Dalgarno, L. Proc. Natl. Acad. Sci. USA, 71:1342, 1974). The spacing between the centre of this putative ribosome-binding site (rbs) and the start codon is 13 bp in comparison to the average spacing of 10 bp in E. coli. The 5' flanking region, upstream of the proposed rbs, shows the presence of possible promoters. The sequences of the -10 region, TAAAAT (151-156), and the -35 region, TTGCTT (127-132), show homology to the consensus of E. coli promoters (Rosenberg, M. and Court, D., Annu. Rev. Genet, 13: 319, 1979) and are identical with promoters recognized

by the E. coli RNA polymerase. The spacing between the putative -10 and -35 sequences is 18 bp, which is comparable with the favoured value of 17 bp.

Between position 1341 and 1359 there is an inverted repeat with the potential to form a stem and loop structure. This repeat does not, however, resemble a typical rho-independent transcription terminator.

Protein D structure

The gene for protein D encodes for a protein of 364 amino acid residues deduced from the nucleotide sequence (Fig. 9). The N-terminal amino acid sequence has typical characteristics of a bacterial lipoprotein signal peptide (Vlasuk et al., J. Biol. Chem. 258:7141, 1983) with its stretch of hydrophilic and basic amino acids at the N-terminus followed by a hydrophobic region of 13 residues, and with a glycin in the hydrophobic core. The putative signal peptide ends with a consensus sequence Leu-Ala-Gly-Cys, recognized by the enzyme signal peptidase II (SpaseII). The primary translation product has a deduced molecular weight of 41,821 daltons. Cleavage by SpaseII would result in a protein of 346 amino acids with a calculated molecular size of 40,068 daltons, in contrast to the estimated size of the mature protein D of approximately 42 kilodaltons. Post-translational modifications of the preprotein may account for this discrepancy. Several attempts to determine the amino-terminal amino acid sequence of protein D were performed by applying about 1000 pmoles thereof in an automated amino acid sequencer. Since no amino acid phenylthiohydantoin derivatives were obtained, the amino-terminal end of the single IgD-receptor polypeptide chain is probably blocked.

Protein D expressed in E. coli JM83 carrying pHIC348 was analysed in immunoblotting experiments (Fig. 10). Cytoplasmic, periplasmic and membrane fractions from cells in late logarithmic phase were separated on a SDS-PAGE gel and electroblotted to an Immobilon filter. A protein that binds all three anti-protein D monoclonal antibodies (16C10, 20G6 and 19B4) and radiolabeled IgD could be detected in all three fractions (lane 2-4) from E. coli JM83/pHIC348 as a single band with an estimated molecular weight of 42 kilodaltons, i.e. equal or similar to protein D prepared from H. influenzae (lane 1, Fig. 10).

The nucleotide sequence and the deduced amino acid sequence of H. influenzae 772 protein D were compared with other proteins of known sequence to determine homology by using a computer search in the EMBL and Genbank Data Libraries. Apart from similarities in the signal sequence no homology was found.

SUMMARY

A novel surface exposed protein of H. influenzae or related Haemophilus species is described. The protein named protein D is an Ig receptor for human IgD and has an apparent molecular weight of 42,000. Protein D can be detected in all of 116 encapsulated and non-encapsulated isolates of H. influenzae studied. The protein from all strains shows in addition to the same apparent molecular weight immunogenic similarities since protein D from all strains interacts with three different mouse monoclonal antibodies and monoclonal human IgD. A method for purification of protein D is described. Cloning of the protein D gene from H. influenzae in E. coli is described as well as the nucleotide sequence and the deduced amino acid sequence corresponding to a molecular weight of 41,821 daltons including a putative signal sequence of 18 amino acids containing a consensus sequence,
Leu-Ala-Gly-Lys for bacterial lipoproteins.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. A surface exposed protein, which is conserved in many strains of Haemophilus influenzae or related Haemophilus species, having an apparent molecular weight of 42,000 and a capacity of binding human IgD, which protein has the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants.

2. A plasmid or phage containing a genetic code for a protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

3. A non-human host containing as plasmid or a phage as defined in claim 2 and capable of producing said protein or variants or a portion of said protein or variants, which host is chosen among bacteria, yeasts and plants.

4. A host according to claim 3, **characterised** in that it is E. coli.

5. A DNA segment comprising a DNA sequence which codes for a protein of Haemophilus influenzae or related Haemophilus species and has the sequence specified in Fig. 9, said protein having an apparent molecular weight of 42,000 and a capacity of binding human IgD, or for naturally occurring or artifi-

cially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

6. A recombinant DNA molecule containing a nucleotide sequence coding for a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants, which nucleotide sequence is fused to another gene.

7. A plasmid or phage containing a fused nucleotide sequence according to claim 6.

8. A non-human host containing at least one plasmid or phage according to claim 7, which host is chosen among bacteria, yeasts or plants.

9. A host according to claim 8, **characterised** in that it is E. coli.

10. A fusion protein or polypeptide in which a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is combined with another protein by the use of a recombinant DNA molecule according to claim 6.

11. A fusion product in which a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capcity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is covalently or by any other means bound to a protein, carbohydrate or matrix.

12. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof.

13. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with another vaccine.

14. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with an immunogenic portion of another molecule.

15. A hybridoma cell capable of producing a monoclonal antibody specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9.

16. A purified antibody which is specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9.

17. A method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with the antibody of claim 16 in the presence of an indicator.

18. A method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with a DNA probe or primer constructed to correspond to the nucleic acids which code for a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

19. A method of detecting IgD using a surface exposed

protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, optionally labelled and/or bound to a matrix.

20. A method of separating IgD using a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, optionally bound to a matrix.

Claims for the following Contracting States : ES, GR

1. A surface exposed protein, which is conserved in many strains of Haemophilus influenzae or related Haemophilus species, having an apparent molecular weight of 42,000 and a capacity of binding human IgD, which protein has the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants.

2. A plasmid or phage containing a genetic code for a protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

3. A non-human host containing as plasmid or a phage as defined in claim 2 and capable of producing said protein or variants or a portion of said protein or variants, which host is chosen among bacteria, yeasts and plants.

4. A host according to claim 3, **characterised** in that it is E. coli.

5. A DNA segment comprising a DNA sequence which codes for a protein of Haemophilus influenzae or related Haemophilus species and has the sequence specified in Fig. 9, said protein having an apparent molecular weight of 42,000 and a capacity of binding human IgD, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

6. A recombinant DNA molecule containing a nucleotide sequence coding for a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants, which nucleotide sequence is fused to another gene.

7. A plasmid or phage containing a fused nucleotide sequence according to claim 6.

8. A non-human host containing at least one plasmid or phage according to claim 7, which host is chosen among bacteria, yeasts or plants.

9. A host according to claim 8, **characterised** in that it is E. coli.

10. A fusion protein or polypeptide in which a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is combined with another protein by the use of a recombinant DNA molecule according to claim 6.

11. A fusion product in which a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capcity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is covalently or by any other means bound to a protein, carbohydrate or matrix.

12. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion

thereof.

13. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with another vaccine.

14. A vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with an immunogenic portion of another molecule.

15. A hybridoma cell capable of producing a monoclonal antibody specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9.

16. A purified antibody which is specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9.

17. A method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with the antibody of claim 16 in the presence of an indicator.

18. A method of detecting the presence of Haemophilus influenzae or related Haemophilus species in a sample by contacting said sample with a DNA probe or primer constructed to correspond to the nucleic acids which code for a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants.

19. A method of detecting IgD using a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, optionally labelled and/or bound to a matrix.

20. A method of separating IgD using a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, optionally bound to a matrix.

21. A method of preparing a surface exposed protein, which is conserved in many strains of Haemophilus influenzae or related Haemophilus species, having an apparent molecular weight of 42,000 and a capacity of binding human IgD, which protein has the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, said method including a conventional preparation step.

22. A method of preparing a plasmid or phage containing a genetic code for a protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants, said method including a conventional preparation step.

23. A method of preparing a non-human host containing as plasmid or a phage as defined in claim 2 and capable of producing said protein or variants or a portion of said protein or variants, which host is chosen among bacteria, yeasts and plants, said method including a conventional preparation step.

24. A method according to claim 23, wherein the host is E. coli.

25. A method of preparing a DNA segment comprising a DNA sequence which codes for a protein of Hae-

mophilus influenzae or related Haemophilus species and has the sequence specified in Fig. 9, said protein having an apparent molecular weight of 42,000 and a capacity of binding human IgD, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants, said method including a conventional preparation step.

26. A method of preparing a recombinant DNA molecule containing a nucleotide sequence coding for a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or for naturally occurring or artificially modified variants thereof having the same function as said protein, or for an immunogenic or IgD-binding portion of said protein or variants, which nucleotide sequence is fused to another gene, said method including a conventional preparation step.

27. A method of preparing a plasmid or phage containing a fused nucleotide sequence according to claim 26, said method including a conventional preparation step.

28. A method of preparing a non-human host containing at least one plasmid or phage prepared according to claim 27, which host is chosen among bacteria, yeasts or plants, said method including a conventional preparation step.

29. A method according to claim 28, wherein the host is E. coli.

30. A method of preparing a fusion protein or polypeptide, wherein a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is combined with another protein by the use of a recombinant DNA molecule prepared according to claim 26.

31. A method of preparing a fusion product wherein a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capcity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion of said protein or variants, is covalently or by any other means bound to a protein, carbohydrate or matrix.

32. A method of preparing a vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, said method including a conventional preparation step.

33. A method of preparing a vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with another vaccine, said method including a conventional preparation step.

34. A method of preparing a vaccine containing a surface exposed protein of Haemophilus influenzae or related Haemophilus species, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, or naturally occurring or artificially modified variants thereof having the same function as said protein, or an immunogenic or IgD-binding portion thereof, combined with an immunogenic portion of another molecule, said method including a conventional preparation step.

35. A method of preparing a hybridoma cell capable of producing a monoclonal antibody specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as described in Fig. 9, said method including a conventional preparation step.

36. A method of preparing a purified antibody which is specific to an immunogenic portion of a surface exposed protein of Haemophilus influenzae or related Haemophilus species, with the proviso that the antibody is not IgD, said protein having an apparent molecular weight of 42,000, a capacity of binding human IgD and the amino acid sequence as de-

scribed in Fig. 9, said method including a conventional preparation step.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Oberflächen-exponiertes Protein, das in vielen Stämmen von Haemophilus influenzae oder verwandten Haemophilus-Species konserviert (aufrechterhalten) wird, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten.

2. Plasmid oder Phage, das (der) einen genetischen Code für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten enthält.

3. Nicht-humaner Wirt, der ein Plasmid oder einen Phagen nach Anspruch 2 enthält und das genannte Protein oder die genannten Varianten oder einen Abschnitt des genannten Proteins oder der genannten Varianten bilden kann und ausgewählt wird aus der Gruppe Bakterien, Hefen und Pflanzen.

4. Wirt nach Anspruch 3, dadurch gekennzeichnet, daß es sich dabei um E. coli handelt.

5. DNA-Segment, das eine DNA-Sequenz aufweist, die die in Fig. 9 dargestellte Sequenz aufweist und für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000 und ein Human-IgD-Bindungsvermögen aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert.

6. Rekombinantes DNA-Molekül, das eine Nucleotid-Sequenz enthält, die für ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert, wobei die Nucleotid-Sequenz mit einem anderen Gen fusioniert ist.

7. Plasmid oder Phage, das (der) eine fusionierte Nucleotid-Sequenz nach Anspruch 6 enthält.

8. Nicht-humaner Wirt, der mindestens ein Plasmid oder einen Phagen nach Anspruch 7 enthält und ausgewählt wird aus der Gruppe Bakterien, Hefen oder Pflanzen.

9. Wirt nach Anspruch 8, dadurch gekennzeichnet, daß es sich dabei um E. coli handelt.

10. Fusionsprotein oder -polypeptid, bei dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten mit einem anderen Protein kombiniert ist durch Verwendung eines rekombinanten DNA-Moleküls nach Anspruch 6.

11. Fusionsprodukt, bei dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten kovalent oder auf irgendeine andere Weise an ein Protein, ein Kohlenhydrat oder eine Matrix gebunden ist.

12. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein

scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon enthält.

13. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem anderen Impfstoff, enthält.

14. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem immunogenen Abschnitt eines anderen Moleküls, enthält.

15. Hybridom-Zelle, die einen monoklonalen Antikörper bilden kann, der spezifisch ist für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species, mit der Maßgabe, daß der Antikörper nicht IgD ist, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist.

16. Gereinigter Antikörper, der für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species spezifisch ist, mit der Maßgabe, daß es sich bei dem Antikörper nicht um IgD handelt, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist.

17. Verfahren zum Nachweis bzw. zur Bestimmung der Anwesenheit von Haemophilus influenzae oder verwandten Haemophilus-Species in einer Probe, bei dem man die genannte Probe mit dem Antikörper gemäß Anspruch 16 in Gegenwart eines Indikators in Kontakt bringt.

18. Verfahren zum Nachweis bzw. zur Bestimmung der Anwesenheit von Haemophilus influenzae oder verwandten Haemophilus-Species in einer Probe, bei dem man die genannte Probe mit einer DNA-Sonde oder einem Starter in Kontakt bringt, der so konstruiert ist, daß er den Nucleinsäuren entspricht, die für ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codieren.

19. Verfahren zum Nachweis bzw. zur Bestimmung von IgD, bei dem man ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten, gegebenenfalls markiert und/oder gebunden an eine Matrix, verwendet.

20. Verfahren zur Abtrennung von IgD, bei dem man ein Oberflächen-expoiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten, gegebenenfalls gebunden an eine Matrix, verwendet.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Oberflächen-exponiertes Protein, das in vielen Stämmen von Haemophilus influenzae oder verwandten Haemophilus-Species konserviert (aufrechterhalten) wird, das ein scheinbares Molekular-

gewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten.

2. Plasmid oder Phage, das (der) einen genetischen Code für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten enthält.

3. Nicht-humaner Wirt, der ein Plasmid oder einen Phagen nach Anspruch 2 enthält und das genannte Protein oder die genannten Varianten oder einen Abschnitt des genannten Proteins oder der genannten Varianten bilden kann und ausgewählt wird aus der Gruppe Bakterien, Hefen und Pflanzen.

4. Wirt nach Anspruch 3, dadurch gekennzeichnet, daß es sich dabei um E. coli handelt.

5. DNA-Segment, das eine DNA-Sequenz aufweist, welche die in Fig. 9 dargestellte Sequenz aufweist und für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000 und ein Human-IgD-Bindungsvermögen aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert.

6. Rekombinantes DNA-Molekül, das eine Nucleotid-Sequenz enthält, die für ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert, wobei die Nucleotid-Sequenz mit einem anderen Gen fusioniert ist.

7. Plasmid oder Phage, das (der) eine fusionierte Nucleotid-Sequenz nach Anspruch 6 enthält.

8. Nicht-humaner Wirt, der mindestens ein Plasmid oder einen Phagen nach Anspruch 7 enthält und ausgewählt wird aus der Gruppe Bakterien, Hefen oder Pflanzen.

9. Wirt nach Anspruch 8, dadurch gekennzeichnet, daß es sich dabei um E. coli handelt.

10. Fusionsprotein oder -polypeptid, in dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten mit einem anderen Protein kombiniert ist durch Verwendung eines rekombinanten DNA-Moleküls nach Anspruch 6.

11. Fusionsprodukt, in dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten kovalent oder auf irgendeine andere Weise an ein Protein, ein Kohlenhydrat oder eine Matrix gebunden ist.

12. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon enthält.

13. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Hu-

man IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem anderen Impfstoff, enthält.

14. Impfstoff, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem immunogenen Abschnitt eines anderen Moleküls, enthält.

15. Hybridom-Zelle, die einen monoklonalen Antikörper bilden kann, der spezifisch ist für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species, mit der Maßgabe, daß der Antikörper nicht IgD ist, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist.

16. Gereinigter Antikörper, der für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species spezifisch ist, mit der Maßgabe, daß es sich bei dem Antikörper nicht um IgD handelt, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist.

17. Verfahren zum Nachweis bzw. zur Bestimmung der Anwesenheit von Haemophilus influenzae oder verwandten Haemophilus-Species in einer Probe, bei dem man die genannte Probe mit dem Antikörper gemäß Anspruch 16 in Gegenwart eines Indikators in Kontakt bringt.

18. Verfahren zum Nachweis bzw. zur Bestimmung der Anwesenheit von Haemophilus influenzae oder verwandten Haemophilus-Species in einer Probe, bei dem man die genannte Probe mit einer DNA-Sonde oder einem Starter in Kontakt bringt, der so konstruiert ist, daß er den Nucleinsäuren entspricht, die für ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codieren.

19. Verfahren zum Nachweis bzw. zur Bestimmung von IgD, bei dem man ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten, gegebenenfalls markiert und/oder gebunden an eine Matrix, verwendet.

20. Verfahren zur Abtrennung von IgD, bei dem man ein Oberflächen-expoiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten, gegebenenfalls gebunden an eine Matrix, verwendet.

21. Verfahren zur Herstellung eines Oberflächen-exponierten Proteins, das in vielen Stämmen von Haemophilus influenzae oder verwandten Haemophilus-Species konserviert (aufrechterhalten) wird, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder von in der Natur vorkommenden oder künstlich modifizierten Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder eines immunogenen oder IgD-bindenden Abschnitts (Teils) des genannten Proteins oder der genannten Varianten, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

22. Verfahren zur Herstellung eines Plasmids oder Phagen, das (der) einen genetischen Code für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Ami-

nosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten enthält, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

23. Verfahren zur Herstellung eines nicht-humanen Wirts, der ein Plasmid oder einen Phagen, wie es (er) in Anspruch 2 definiert ist, enthält und der das genannte Protein oder die genannten Varianten oder einen Abschnitt des genannten Proteins oder der genannten Varianten bilden kann und ausgewählt wird aus der Gruppe Bakterien, Hefen und Pflanzen, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

24. Verfahren nach Anspruch 23, wobei es sich bei dem Wirt um E. coli handelt.

25. Verfahren zur Herstellung eines DNA-Segments, das eine DNA-Sequenz aufweist, welche die in Fig. 9 dargestellte Sequenz aufweist und für ein Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000 und ein Human-IgD-Bindungsvermögen aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

26. Verfahren zur Herstellung eines rekombinanten DNA-Moleküls, das eine Nucleotid-Sequenz enthält, die für ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, wobei das Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder für in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das Protein haben, oder für einen immunogenen oder IgD-bindenden Abschnitt des genannten Proteins oder der genannten Varianten codiert, wobei die Nucleotid-Sequenz mit einem anderen Gen fusioniert ist, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

27. Verfahren zur Herstellung eines Plasmids oder Phagen, das (der) eine fusionierte Nucleotid-Sequenz nach Anspruch 26 enthält, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

28. Verfahren zur Herstellung eines nicht-humanen Wirts, der mindestens ein Plasmid oder einen Phagen, hergestellt nach Anspruch 27 enthält, und ausgewählt wird aus der Gruppe Bakterien, Hefen oder Pflanzen, wobei das Verfahren eine nicht-konventionelle Herstellungsstufe umfaßt.

29. Verfahren nach Anspruch 28, worin der Wirt E. coli ist.

30. Verfahren zur Herstellung eines Fusionsproteins oder Polypeptids, bei dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten mit einem anderen Protein kombiniert wird durch Verwendung eines nach Anspruch 26 hergestellten rekombinanten DNA-Moleküls.

31. Verfahren zur Herstellung eines Fusionsprodukts, bei dem ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder ein immunogener oder IgD-bindender Abschnitt des genannten Proteins oder der genannten Varianten kovalent oder auf irgendeine andere Weise an ein Protein, ein Kohlenhydrat oder eine Matrix gebunden wird.

32. Verfahren zur Herstellung eines Impfstoffs, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein HumanIgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon enthält, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

33. Verfahren zur Herstellung eines Impfstoffs, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000,

ein Human IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem anderen Impfstoff, enthält, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

34. Verfahren zur Herstellung eines Impfstoffs, der ein Oberflächen-exponiertes Protein von Haemophilus influenzae oder verwandten Haemophilus-Species, das ein scheinbares Molekulargewicht von 42 000, ein Human IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, oder in der Natur vorkommende oder künstlich modifizierte Varianten davon, welche die gleiche Funktion wie das genannte Protein haben, oder einen immunogenen oder IgD-bindenden Abschnitt davon, kombiniert mit einem immunogenen Abschnitt eines anderen Moleküls, enthält, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

35. Verfahren zur Herstellung einer Hybridom-Zelle, die einen monoklonalen Antikörper bilden kann, der spezifisch ist für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species, mit der Maßgabe, daß der Antikörper nicht IgD ist, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

36. Verfahren zur Herstellung eines gereinigten Antikörpers, der für einen immunogenen Abschnitt eines Oberflächen-exponierten Proteins von Haemophilus influenzae oder verwandten Haemophilus-Species spezifisch ist, mit der Maßgabe, daß es sich bei dem Antikörper nicht um IgD handelt, wobei das genannte Protein ein scheinbares Molekulargewicht von 42 000, ein Human-IgD-Bindungsvermögen und die in Fig. 9 dargestellte Aminosäure-Sequenz aufweist, wobei das Verfahren eine konventionelle Herstellungsstufe umfaßt.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Protéine exposée à la surface, qui est conservée dans de nombreuses souches de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ayant une masse moléculaire apparente de 42 000 et une capacité à lier l'IgD humaine, laquelle protéine a la séquence d'acides aminés telle que décrite sur la figure 9, ou variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes.

2. Plasmide ou phage contenant un code génétique pour une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou pour des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou pour une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes.

3. Hôte non humain contenant un plasmide ou un phage tel que défini dans la revendication 2 et capable de produire ladite protéine ou lesdites variantes ou une portion de ladite protéine ou desdites variantes, lequel hôte est choisi parmi les bactéries, les levures et les végétaux.

4. Hôte selon la revendication 3, caractérisé en ce qu'il s'agit de E. coli.

5. Fragment d'ADN comprenant une séquence d'ADN qui code une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées et qui a la séquence spécifiée sur la figure 9, ladite protéine ayant une masse moléculaire apparente de 42 000 et une capacité à lier l'IgD humaine, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes.

6. Molécule d'ADN recombiné contenant une séquence nucléotidique codant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, laquelle séquence nucléotidique est fusionnée à un autre gène.

7. Plasmide ou phage contenant une séquence nucléotidique fusionnée selon la revendication 6.

8. Hôte non humain contenant au moins un plasmide ou phage selon la revendication 7, lequel hôte est choisi parmi les bactéries, les levures ou les végétaux.

9. Hôte selon la revendication 8, caractérisé en ce qu'il s'agit de E. coli.

10. Protéine ou polypeptide de fusion où une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est combinée avec une autre protéine au moyen d'une molécule d'ADN recombiné selon la revendication 6.

11. Produit de fusion dans lequel une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est liée de manière covalente ou par tout autre moyen à une protéine, à un glucide ou à une matrice.

12. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci.

13. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec un autre vaccin.

14. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec une portion immunogène d'une autre molécule.

15. Cellule d'hybridome capable de produire un anticorps monoclonal spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9.

16. Anticorps purifié qui est spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9.

17. Procédé de détection de la présence de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées dans un échantillon par mise en contact dudit échantillon avec l'anticorps selon la revendication 16 en présence d'un indicateur.

18. Procédé de détection de la présence de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées dans un échantillon par mise en contact dudit échantillon avec une sonde ou amorce d'ADN construite pour correspondre aux acides nucléiques qui codent une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes.

19. Procédé de détection d'IgD au moyen d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou de variantes naturelles ou modifiées ar-

tificiellement de celle-ci ayant la même fonction que ladite protéine, ou d'une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, éventuellement marquée et/ou liée à une matrice.

20. Procédé de séparation d'IgD au moyen d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou de variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou d'une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, liée éventuellement à une matrice.

Revendications pour les Etats contractants suivants : ES, GR

1. Protéine exposée à la surface, qui est conservée dans de nombreuses souches de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ayant une masse moléculaire apparente de 42000 et une capacité à lier l'IgD humaine, laquelle protéine a la séquence d'acides aminés telle que décrite sur la figure 9, ou variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou portion immunogène ou liant IgD de ladite protéine ou desdites variantes.

2. Plasmide ou phage contenant un code génétique pour une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou pour des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou pour une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes.

3. Hôte non humain contenant un plasmide ou un phage tel que défini dans la revendication 2 et capable de produire ladite protéine ou lesdites variantes ou une portion de ladite protéine ou desdites variantes, lequel hôte est choisi parmi les bactéries, les levures et les végétaux.

4. Hôte selon la revendication 3, caractérisé en ce qu'il s'agit de E. coli.

5. Fragment d'ADN comprenant une séquence d'ADN qui code une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées et qui a la séquence spécifiée sur la figure 9, ladite protéine ayant une masse moléculaire apparente de 42 000 et une capacité à lier l'IgD humaine, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes.

6. Molécule d'ADN recombiné contenant une séquence nucléotidique codant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, laquelle séquence nucléotidique est fusionnée à un autre gène.

7. Plasmide ou phage contenant une séquence nucléotidique fusionnée selon la revendication 6.

8. Hôte non humain contenant au moins un plasmide ou phage selon la revendication 7, lequel hôte est choisi parmi les bactéries, les levures ou les végétaux.

9. Hôte selon la revendication 8, caractérisé en ce qu'il s'agit de E. coli.

10. Protéine ou polypeptide de fusion où une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est combinée avec une autre protéine au moyen d'une molécule d'ADN recombiné selon la revendication 6.

11. Produit de fusion dans lequel une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est liée de manière covalente ou par tout autre moyen à une protéine, à un glucide ou à une matrice.

12. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci.

13. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec un autre vaccin.

14. Vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec une portion immunogène d'une autre molécule.

15. Cellule d'hybridome capable de produire un anticorps monoclonal spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9.

16. Anticorps purifié qui est spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9.

17. Procédé de détection de la présence de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées dans un échantillon par mise en contact dudit échantillon avec l'anticorps selon la revendication 16 en présence d'un indicateur.

18. Procédé de détection de la présence de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées dans un échantillon par mise en contact dudit échantillon avec une sonde ou amorce d'ADN construite pour correspondre aux acides nucléiques qui codent une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes.

19. Procédé de détection d'IgD au moyen d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou de variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou d'une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, éventuellement marquée et/ou liée à une matrice.

20. Procédé de séparation d'IgD au moyen d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou de variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou d'une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, liée éventuellement à une matrice.

21. Procédé de préparation d'une protéine exposée à la surface, qui est conservée dans de nombreuses souches de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ayant une masse moléculaire apparente de 42 000 et une capacité à lier l'IgD humaine, laquelle protéine a la séquence d'acides aminés telle que décrite sur la figure 9, ou variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou portion immunogène ou liant IgD de ladite protéine ou desdites variantes, ledit procédé comprenant une étape de préparation conventionnelle.

22. Procédé de préparation d'un plasmide ou phage contenant un code génétique pour une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à

lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou pour des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou pour une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes, ledit procédé comprenant une étape de préparation conventionnelle.

23. Procédé de préparation d'un hôte non humain contenant un plasmide ou un phage tel que défini dans la revendication 2 et capable de produire ladite protéine ou lesdites variantes ou une portion de ladite protéine ou desdites variantes, lequel hôte est choisi parmi les bactéries, les levures et les végétaux, ledit procédé comprenant une étape de préparation conventionnelle.

24. Procédé selon la revendication 23, dans lequel l'hôte est E. coli.

25. Procédé de préparation d'un segment d'ADN comprenant une séquence d'ADN qui code une protéine de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées et qui a la séquence spécifiée sur la figure 9, ladite protéine ayant une masse moléculaire apparente de 42 000 et une capacité à lier l'IgD humaine, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant l'IgD de ladite protéine ou desdites variantes, ledit procédé comprenant une étape de préparation conventionnelle.

26. Procédé de préparation d'une molécule d'ADN recombiné contenant une séquence nucléotidique codant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, laquelle séquence nucléotidique est fusionnée à un autre gène, ledit procédé comprenant une étape de préparation conventionnelle.

27. Procédé de préparation d'un plasmide ou phage contenant une séquence nucléotidique fusionnée selon la revendication 26, ledit procédé comprenant une étape de préparation conventionnelle.

28. Procédé de préparation d'un hôte non humain contenant au moins un plasmide ou phage préparé selon la revendication 27, lequel hôte est choisi parmi les bactéries, les levures ou les végétaux, ledit procédé comprenant une étape de préparation conventionnelle.

29. Procédé selon la revendication 28, dans lequel l'hôte est E. coli.

30. Procédé de préparation d'une protéine ou polypeptide de fusion où une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est combinée avec une autre protéine au moyen d'une molécule d'ADN recombiné selon la revendication 6.

31. Procédé de préparation d'un produit de fusion dans lequel une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de ladite protéine ou desdites variantes, est liée de manière covalente ou par tout autre moyen à une protéine, à un glucide ou à une matrice.

32. Procédé de préparation d'un vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, ledit procédé comprenant une étape de préparation conventionnelle.

33. Procédé de préparation d'un vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec un autre vaccin, ledit procédé comprenant une étape de préparation conventionnelle.

34. Procédé de préparation d'un vaccin contenant une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ou des variantes naturelles ou modifiées artificiellement de celle-ci ayant la même fonction que ladite protéine, ou une portion immunogène ou liant IgD de celle-ci, en combinaison avec une portion immunogène d'une autre molécule, ledit procédé comprenant une étape de préparation conventionnelle.

35. Procédé de préparation d'une cellule d'hybridome capable de produire un anticorps monoclonal spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ledit procédé comprenant une étape de préparation conventionnelle.

36. Procédé de préparation d'un anticorps purifié qui est spécifique d'une portion immunogène d'une protéine exposée à la surface de Haemophilus influenzae ou d'espèces d'Haemophilus apparentées, à condition que l'anticorps ne soit pas IgD, ladite protéine ayant une masse moléculaire apparente de 42 000, une capacité à lier l'IgD humaine et la séquence d'acides aminés telle que décrite sur la figure 9, ledit procédé comprenant une étape de préparation conventionnelle.

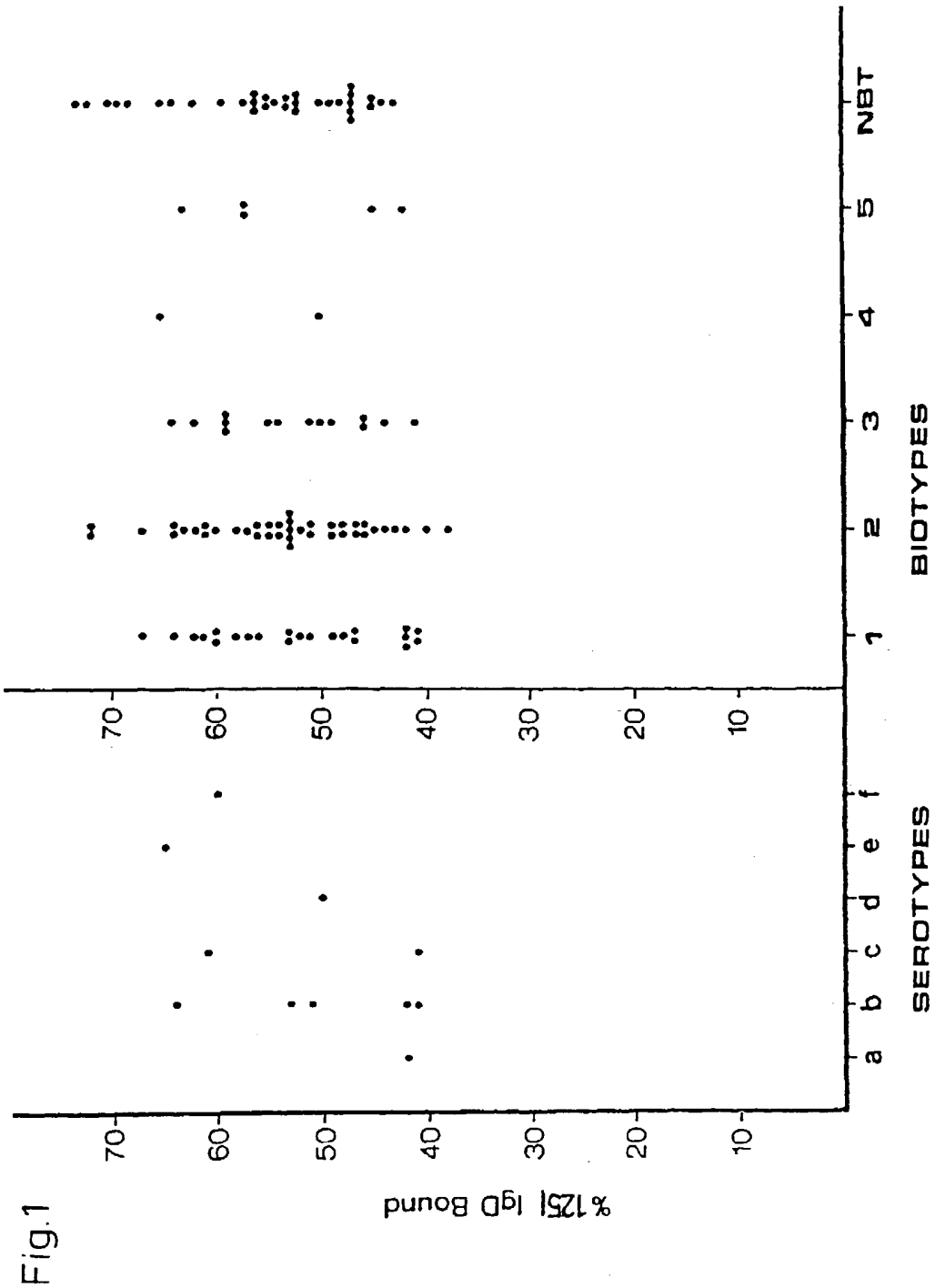
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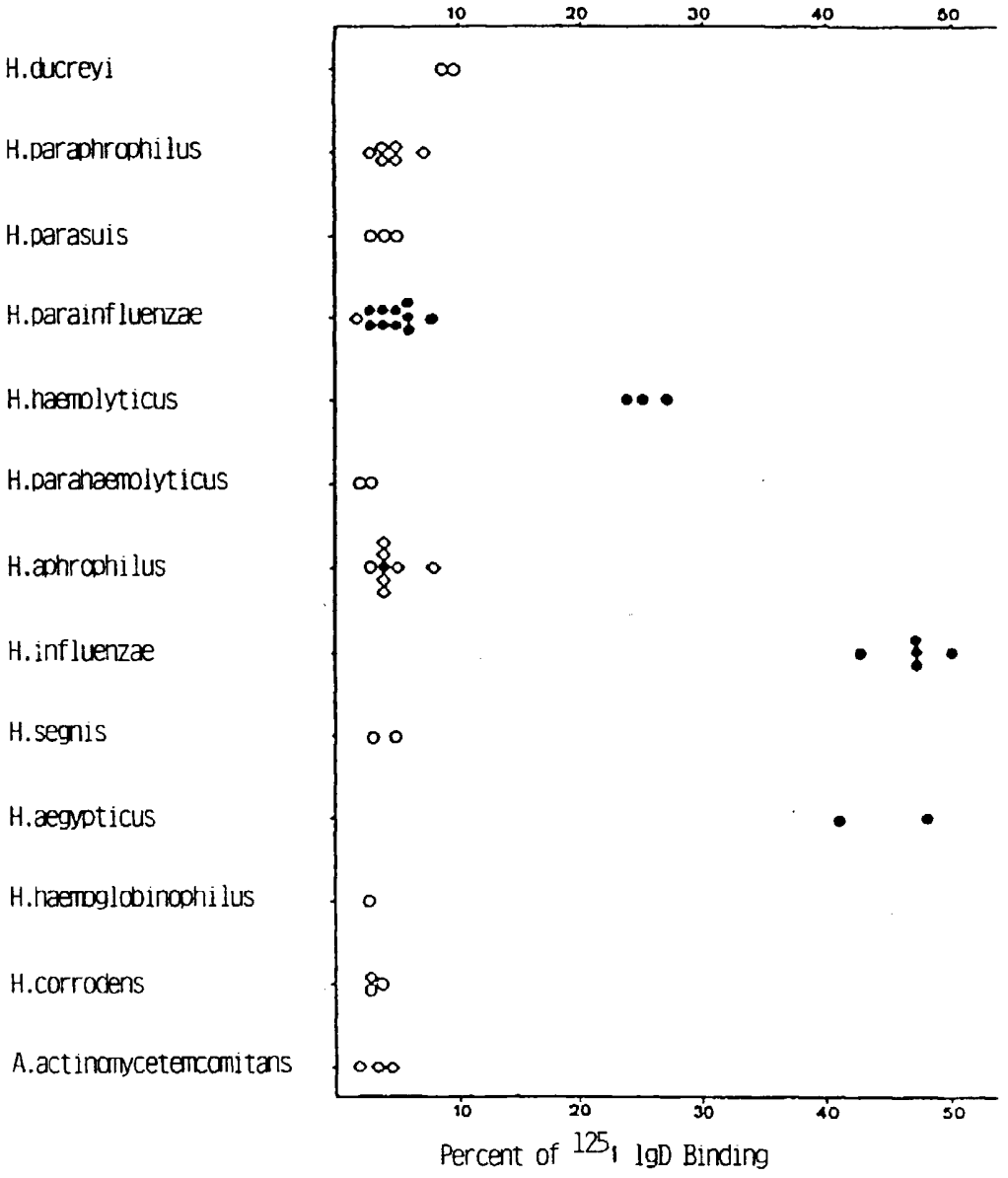
Fig.1

Fig.2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40
50
Percent of 125I IgD Binding

Fig.3

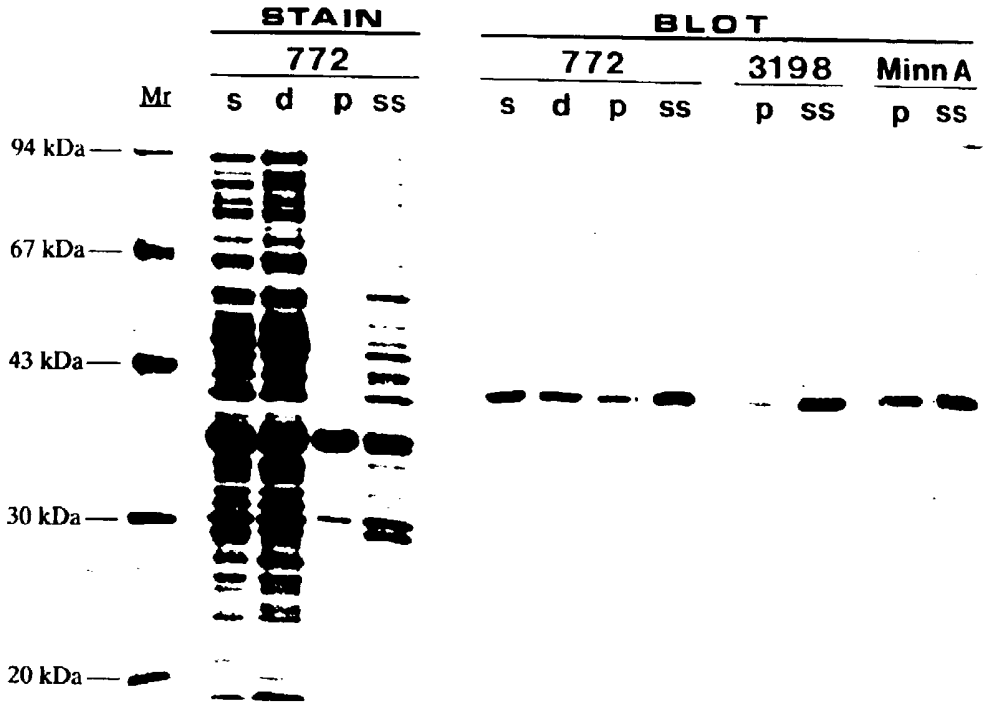

STAIN
772
BLOT
772
3198
Minn A
Mr
s d p ss
s d p ss
p ss
p ss
94 kDa
67 kDa
43 kDa
30 kDa
20 kDa

Fig.4

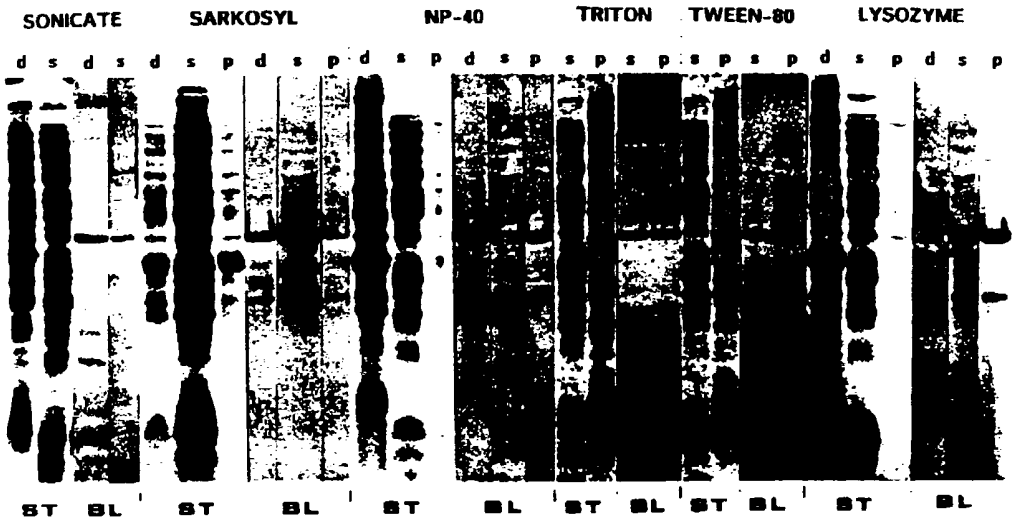

SONICATE
SARKOSYL
NP-40
TRITON
TWEEN-80
LYSOZYME
d s d s d s p d s p d s p d s p s p s p s p d s p d s p
ST BL ST BL ST BL ST BL ST BL ST BL

Fig.5

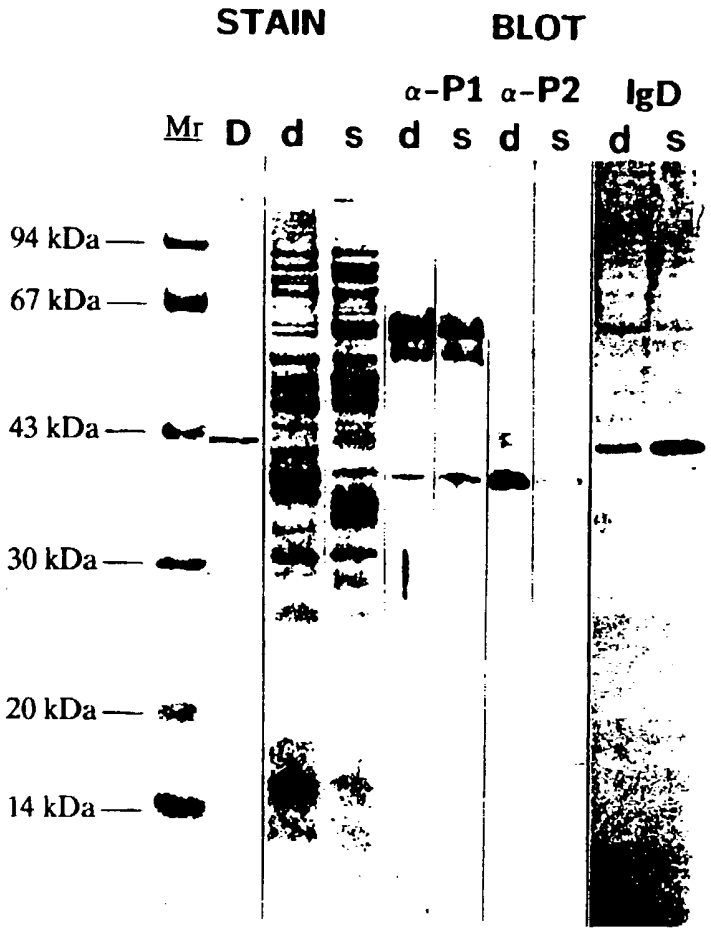

STAIN
BLOT
α-P1
α-P2
IgD
Mr
D
d
s
d
s
d
s
d
s
94 kDa
67 kDa
43 kDa
30 kDa
20 kDa
14 kDa

Fig.6

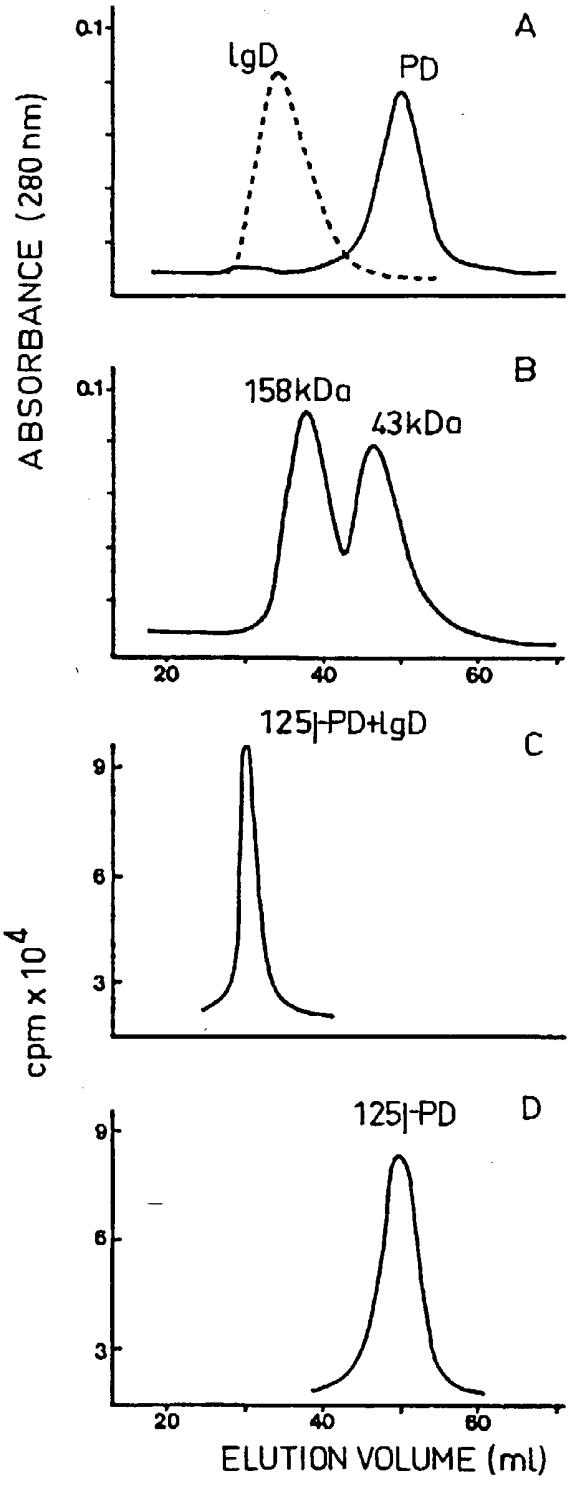
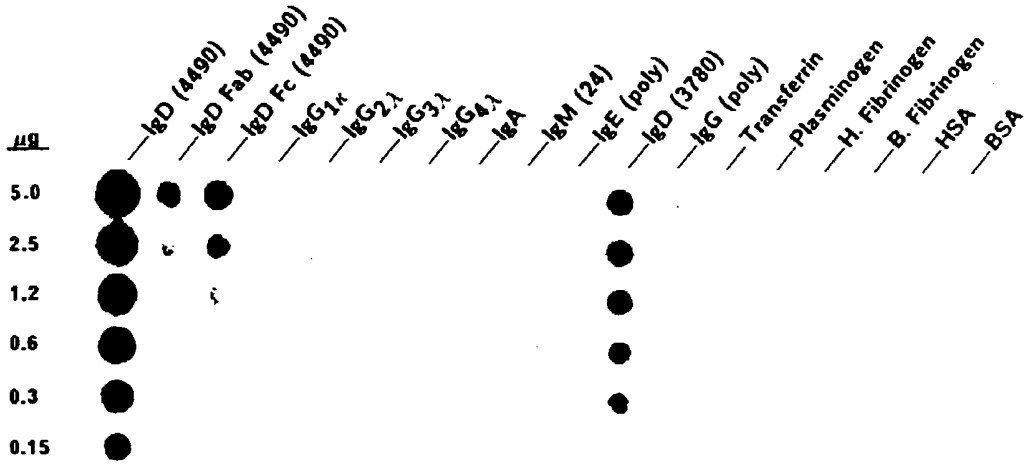

A
0.1
IgD
PD
ABSORBANCE (280 nm)
B
0.1
158kDa
43kDa
20
40
60
125I-PD+IgD
C
9
6
3
cpm x 10^4
125I-PD
D
9
6
3
20
40
60
ELUTION VOLUME (ml)

Fig.7


µg
5.0
2.5
1.2
0.6
0.3
0.15
IgD (4490)
IgD Fab (4490)
IgD Fc (4490)
IgG1κ
IgG2λ
IgG3λ
IgG4λ
IgA
IgM (24)
IgE (poly)
IgD (3780)
IgG (poly)
Transferrin
Plasminogen
H. Fibrinogen
B. Fibrinogen
HSA
BSA

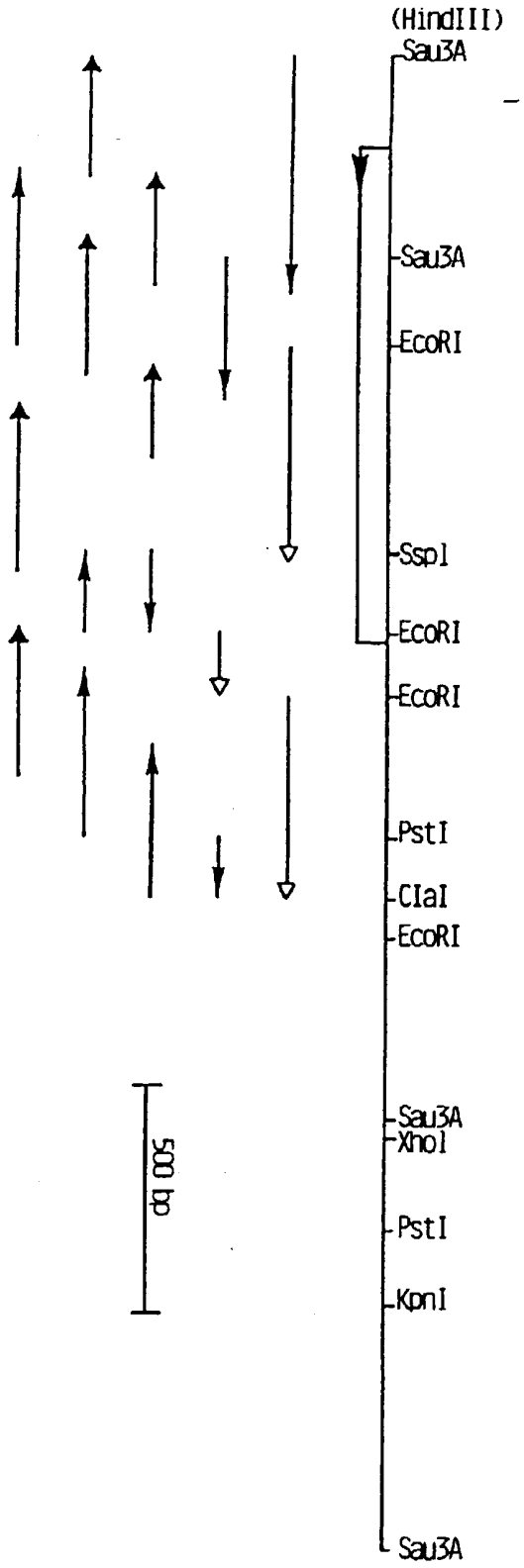

(HindIII)
Sau3A
Sau3A
EcoRI
SspI
EcoRI
EcoRI
PstI
ClaI
EcoRI
Sau3A
XhoI
PstI
KpnI
Sau3A
500 bp

Fig.8

Fig.9a

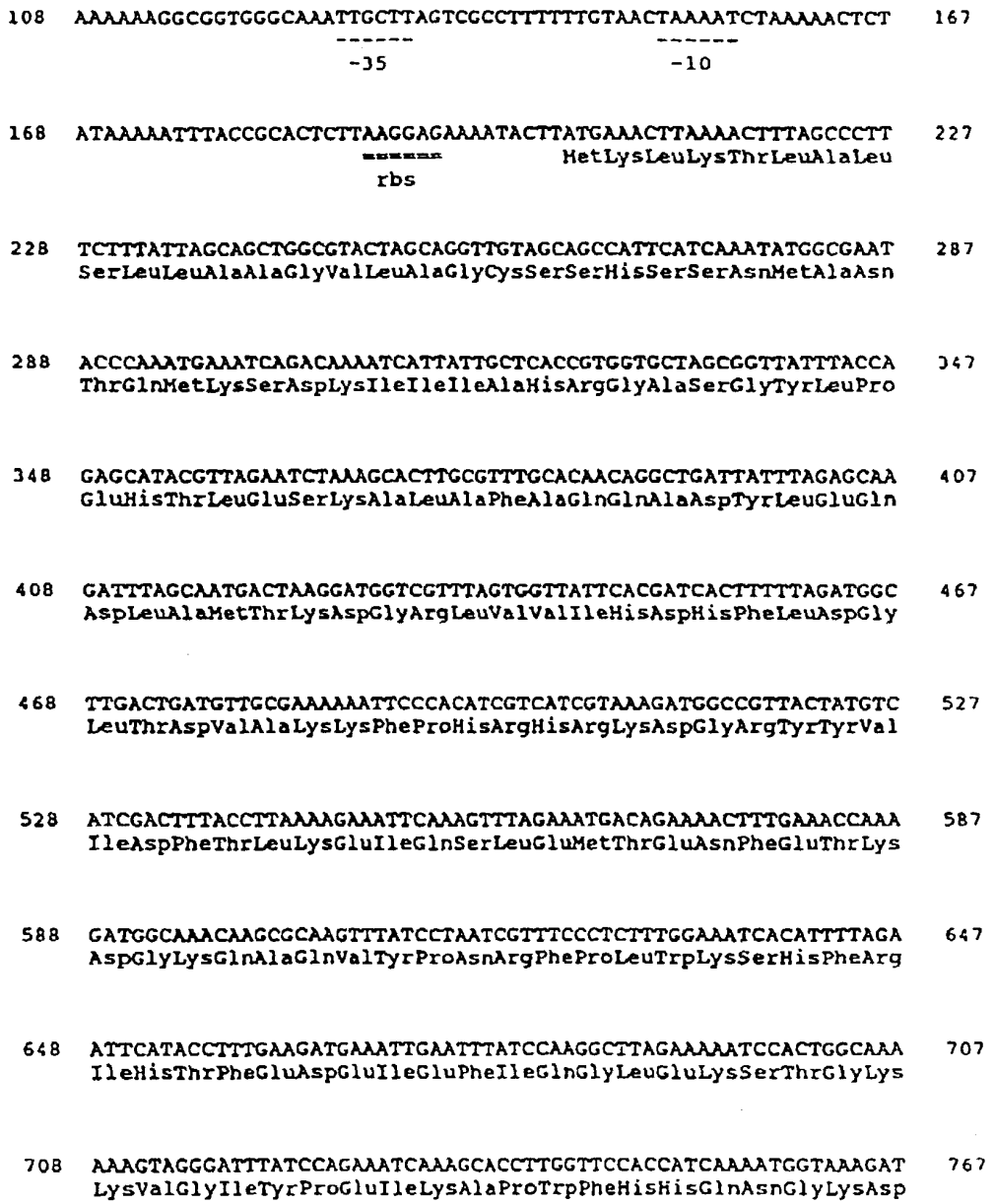
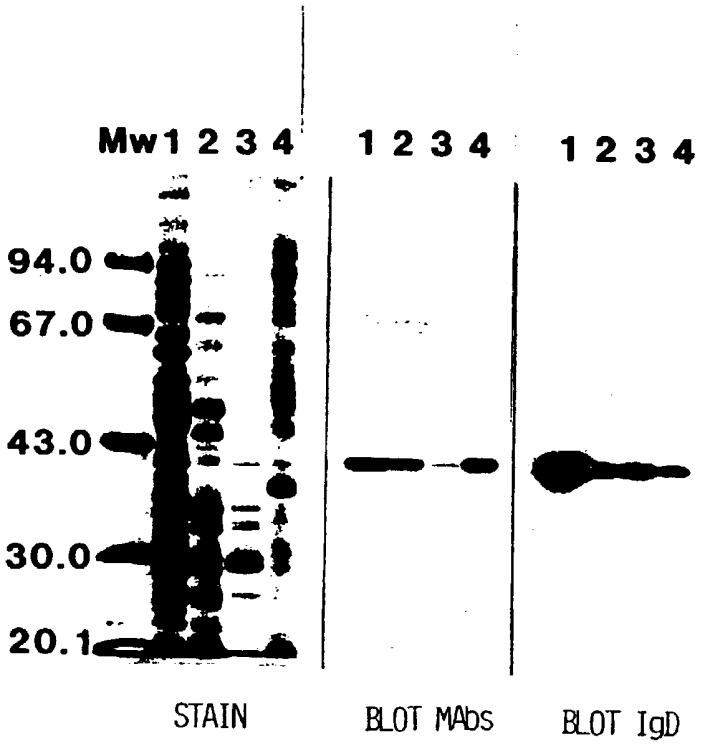

108 AAAAAAGGCGGTGGGCAAATTGCTTAGTCGCCTTTTTTGTAACTAAAATCTAAAAACTCT 167
-35
-10
168 ATAAAAATTTACCGCACTCTTAAGGAGAAAATACTTATGAAACTTAAAACTTTAGCCCTT 227
rbs
MetLysLeuLysThrLeuAlaLeu
228 TCTTTATTAGCAGCTGGCGTACTAGCAGGTTGTAGCAGCCATTCATCAAATATGGCGAAT 287
SerLeuLeuAlaAlaGlyValLeuAlaGlyCysSerSerHisSerSerAsnMetAlaAsn
288 ACCCAAATGAAATCAGACAAAATCATTATTGCTCACCGTGGTGCTAGCGGTTATTTACCA 347
ThrGlnMetLysSerAspLysIleIleIleAlaHisArgGlyAlaSerGlyTyrLeuPro
348 GAGCATACGTTAGAATCTAAAGCACTTGCGTTTGCACAACAGGCTGATTATTTAGAGCAA 407
GluHisThrLeuGluSerLysAlaLeuAlaPheAlaGlnGlnAlaAspTyrLeuGluGln
408 GATTTAGCAATGACTAAGGATGGTCGTTTAGTGGTTATTCACGATCACTTTTTAGATGGC 467
AspLeuAlaMetThrLysAspGlyArgLeuValValIleHisAspHisPheLeuAspGly
468 TTGACTGATGTTGCGAAAAAATTCCCACATCGTCATCGTAAAGATGGCCGTTACTATGTC 527
LeuThrAspValAlaLysLysProProHisArgHisArgLysAspGlyArgTyrTyrVal
528 ATCGACTTTACCTTAAAAGAAATTCAAAGTTTAGAAATGACAGAAAACTTTGAAACCAAA 587
IleAspPheThrLeuLysGluIleGlnSerLeuGluMetThrGluAsnPheGluThrLys
588 GATGGCAAACAAGCGCAAGTTTATCCTAATCGTTTCCCTCTTTGGAAATCACATTTTAGA 647
AspGlyLysGlnAlaGlnValTyrProAsnArgPheProLeuTrpLysSerHisPheArg
648 ATTCATACCTTTGAAGATGAAATTGAATTTATCCAAGGCTTAGAAAAATCCACTGGCAAA 707
IleHisThrPheGluAspGluIleGluPheIleGlnGlyLeuGluLysSerThrGlyLys
708 AAAGTAGGGATTTATCCAGAAATCAAAGCACCTTGGTTCCACCATCAAAATGGTAAAGAT 767
LysValGlyIleTyrProGluIleLysAlaProTrpPheHisHisGlnAsnGlyLysAsp

Fig.9b

```
768  ATTGCTGCTGAAACGCTCAAAGTGTTAAAAAAATATGGCTATGATAAGAAAACCGATATG  827
     IleAlaAlaGluThrLeuLysValLeuLysLysTyrGlyTyrAspLysLysThrAspMet

828  GTTTACTTACAAACTTTCGATTTTAATGAATTAAAACGTATCAAAACGGAATTACTTCCA  887
     ValTyrLeuGlnThrPheAspPheAsnGluLeuLysArgIleLysThrGluLeuLeuPro

888  CAAATGGGAATGGATTTGAAATTAGTTCAATTAATTGCTTATACAGATTGGAAAGAAACA  947
     GlnMetGlyMetAspLeuLysLeuValGlnLeuIleAlaTyrThrAspTrpLysGluThr

948  CAAGAAAAAGACCCAAAGGGTTATTGGGTAAACTATAATTACGATTGGATGTTTAAACCT  1007
     GlnGluLysAspProLysGlyTyrTrpValAsnTyrAsnTyrAspTrpMetPheLysPro

1008 GGTGCAATGGCAGAAGTGGTTAAATATGCCGATGGTGTTGGCCCAGGTTGGTATATGTTA  1067
     GlyAlaMetAlaGluValValLysTyrAlaAspGlyValGlyProGlyTrpTyrMetLeu

1068 GTTAATAAAGAAGAATCCAAACCTGATAATATTGTGTACACTCCGTTGGTAAAAGAACTT  1127
     ValAsnLysGluGluSerLysProAspAsnIleValTyrThrProLeuValLysGluLeu

1128 GCACAATATAATGTGGAAGTGCATCCTTACACCGTGCGTAAAGATGCACTGCCCGAGTTT  1187
     AlaGlnTyrAsnValGluValHisProTyrThrValArgLysAspAlaLeuProGluPhe

1188 TTCACAGACGTAAATCAAATGTATGATGCCTTATTGAATAAATCAGGGGCAACAGGTGTA  1247
     PheThrAspValAsnGlnMetTyrAspAlaLeuLeuAsnLysSerGlyAlaThrGlyVal

1248 TTTACTGATTTCCCAGATACTGGCGTGGAATTCTTAAAAGGAATAAAATAATATCCCTCA  1307
     PheThrAspPheProAspThrGlyValGluPheLeuLysGlyIleLysEnd

1308 CAACCGTGGGTAAACATACCCACGGTTAACTAGGTTTCTATATCGTAGAAACTAAAAATC  1367
```

Fig.10



1. H.influenzae
2. E.coli pHIC348 (cytoplasmic fraction)
3. E.coli pHIC348 (periplasmic fraction)
4. E.coli pHIC348 (membrane fraction)